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The
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THE ISOLATION AND IDENTIFICATION OF ENTERIC BACTERIA*

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The isolation and identification of organisms of the Salmonella-Shigella group is a constant problem for the laboratory of an institution where thousands of individuals are housed. This search for carriers of enteric pathogens is carried out on second or third post-cathartic stool specimens to which urine has been added.[1] Two such specimens are run on all new patients and empoyees, and three are done if they are to be food handlers. An average of about 300 cultures a month are handled. Up to the present time, in this laboratory, 30,000 specimens have been cultured by the method to be described.

MATERIALS AND METHODS

It is generally understood when culturing for the gram-negative bacilli inhabiting the gastro-intestinal tract of man, that it is better not to rely upon any one medium but to employ several.[2,3,4] Therefore, the specimens are streaked with a large loop (22 gauge, 1 cm. in dia.) onto Hajna-Perry modification of Wilson-Blair, Shigella-Salmonella Agar and Eosin Methylene Blue Agar. The plates are streaked in such a manner that isolated colonies are secured. Depending upon the consistency of the specimen, about five loopfuls are streaked on the HP, three on the SS, and one on the EMB. A Selenite enrichment tube is also inoculated with approximately one gram of feces. After twenty four hours incubation, suspicious colonies are picked from the SS and EMB Agars, and material from the Selenites is streaked to an SS plate. On the third day, the HP and the indirect SS plates are picked.

Plating Media

The formula of Wilson and Blair[5] as modified by Hajna and Perry is a highly selective, as well as differential, medium for

* Third ASMT award, read before convention, Louisville, Ky., June 1953.

the isolation of Salmonella typhosa. It is also satisfactory for other members of the Salmonella group. Gram-positive bacteria and coliform bacilli are inhibited. This permits the use of large inoculum which increases the possibility of isolating the organisms. However, the plates must be streaked in a manner that will give discrete colonies on some part of them. Too heavy an inoculum causes blackening of the entire plate.

The isolated colony of S. typhosa grows into the medium in a cone shape. It is black and is surrounded by a black or brownish-black zone that may be several times the size of the colony. By reflected light this zone has a metallic sheen. Blackening is due to formation of the sulfides of bismuth and iron, since S. typhosa in the presence of a fermentable carbohydrate is able to reduce a sulfite to a sulfide. Sodium phosphate absorbs the acids, produced in fermentation of glucose, and insures blackening of the colony with iron sulfide. Brilliant green intensifies the blackness and suppresses the coli. The excess of sodium sulfite also inhibits the coliform organisms. Plates that are overcrowded will not show this reaction. In congested areas the colonies may appear light green.

On this medium coli is usually completely inhibited. Some strains will develop small black, brown, or greenish surface colonies. The color is confined to the colony. Some may be similar in appearance to typhoid organisms but of course are easily differentiated by the use of other media. A few strains of Shigella will grow although the brilliant green usually suppresses them.

SS Agar, as the name indicates, is intended for the isolation of Shigella and Salmonella organisms. Using neutral red as an indicator, it differentiates quite clearly between lactose fermenters and lactose non-fermenters. It is a thiosulfate-citrate-bile agar that greatly inhibits coliform organisms without restricting the growth of pathogenic gram-negative bacilli.

The lactose fermenting organisms, that do develop on the medium, form very characteristic deep pink colored colonies. These at times are surrounded by a zone of precipitated bile. This reaction is due to the action of the acids, produced by fermentation of lactose, upon the bile salts and the absorption of neutral red.

Opaque, transparent and translucent uncolored colonies may be those of Salmonella, Shigella, slowly growing or slow lactose fermenting strains of Escherichia or Aerobactor, Paracolobactrum, Proteus, Pseudomonas, or Alcaligenes. Some Proteus and Salmonella types may produce black centered colonies. Its differential qualities are demonstrated when colonies of several species are found on a single plate.

EMB Agar, a non-inhibitive differential plate medium, is included to pick up the delicate strains of Salmonella and Shigella that do not grow well on the selective media. This medium shows a distinct

difference between colonies of lactose and sucrose fermenting bacteria and those that do not ferment these sugars.

Colonies of Escherichia coli are deep reddish-purple and have a metallic sheen that is best seen when they are examined with reflected light. Aerobactor aerogenes produce convex purple colonies with a mucoid periphery and a sheen to the center. The non-fermenters remain uncolored, and small colorless colonies may be any of the gram-negative or gram-positive organisms that are found in the intestinal tract. The gram-positives may also develop pin point colonies that have a sheen.

Enrichment

The selenium salts inhibit, to a certain extent, the growth of E. coli. A broth containing sodium acid selenite may be used as an enrichment medium for the isolation of S. typhosa and other members of the Salmonella group. Leifson[6] demonstrated that the number of colon bacilli decreases during the first eight to twelve hours in the medium followed by an increase. He further showed that the typhoid bacilli multiply fairly rapidly from the start and soon greatly outnumber the coli, assuming equal numbers in the beginning. The layer of medium should be two inches or more in depth, since a high oxygen tension leads to a more rapid destruction of S. typhosa.

Shigella and Alcaligenes are inhibited. Proteus and Pseudomonas are not inhibited. In fact, the Proteus group tend to increase in numbers when incubated in enrichment media used for suppressing E. coli.

Screening

When suspicious colonies are found on the various plates, at least two of each type are picked for identification. As a preliminary step, in determining whether or not these colonies are composed of pathogenic organisms, they are inoculated into tubes of Lead Semisolid Agar, a medium developed by Friewer and Shaughnessy.[7] Motility, hydrogen sulfide production, and lactose fermentation can all be determined by use of this tube medium. Its reactions are clear cut and it is a great saver of time for the laboratory with a large volume of work. The cultures may be read in 12-24 hours. The medium is inoculated by a stab with a straight needle. This inoculation can be made from a single colony or from a broth or agar sub-culture. The stab should go about one half the depth of the medium in the tube.

Tittsler and Sandholzer[8] pointed out that the results were macroscopic and cumulative when semisolid agar was used for the detection of motility. This eliminates the difficulty encountered with direct microscopic examination when only a few cells in a culture remain motile. Semisolid agar is much simpler than a hanging drop when large numbers of cultures are handled.

After incubation a motile culture shows a diffusion of the growth

from the place where it was deposited in the tube, while a non-motile one shows no diffusion from the line of inoculation. Cultures vary in the amount of motility. For example, S. typhosa does not diffuse through the medium as rapidly as the other members of the Salmonella group or members of the Proteus group. Pseudomonas and Alcaligenes are obligate aerobes and grow only at the surface of semisolid agar, therefore the motility of these organisms cannot be read with this medium.

Hydrogen sulfide production is indicated by a brown color, since the medium contains lead acetate.

Lactose fermentation is demonstrated by a yellow color. Brom thymol blue is the indicator which is yellow in acid range. If gas is formed, there will be small bubbles near the surface or throughout the medium.

When the three functions of Lead Semisolid Agar are considered collectively, its usefulness as a screening test is made clear. Several species of organisms can be discarded at this point. It rules out lactose fermenting colonies that are picked from plating media, since none of the enteric pathogens ferment lactose within twenty-four hours. Strains that grow at the surface of the medium only are suspected Pseudomonas or Alcaligenes and these are inoculated into dextrose. This carbohydrate is chosen because all the intestinal pathogens ferment it. Alcaligenes faecales ferments none of the usual carbohydrates. Pseudomonas aeruginosa does not ferment the carbohydrates except for an occasional strain that produces a slight reaction in dextrose. If dextrose is fermented, place the organism in sugars as described later for Shigella. All non-motile strains are checked by Gram's stain. Gram-positive cultures are discarded.

Further Tests

The gram-negative, non-motile, non-hydrogen sulfide producing cultures are possible Shigella organisms, and tubes containing non-lactose fermenting, motile bacteria that produce hydrogen sulfide belong to the Proteus or Salmonella groups. These are inoculated into dextrose, mannite, and sucrose broth and peptone water for an indol test.

The Shigella all ferment dextrose, some ferment mannite, and with few exceptions none produce gas from carbohydrates. Lactose is fermented by some but only after prolonged incubation. A few are motile and a few may produce hydrogen sulfide. The Shigella fall roughly into three types: non-mannite and non-lactose fermenters, mannite fermenters and non-lactose fermenters, and mannite and lactose fermenters.

With few exceptions the typical Salmonella reactions are acid and gas in dextrose and mannite, sucrose and lactose negative, and indol negative. Hydrogen sulfide is usually produced. A few are non-motile. Antigenic studies have given evidence that the typhoid

bacillus belongs in the genus Salmonella,[9] but it is helpful to remember that S. typhosa does not produce gas in carbohydrate media, thus differentiating it from most of the members of the group.

Members of the genus Proteus do not ferment mannite. Some ferment sucrose and some do not. They vary in the ability to produce indol.

If there is any doubt as to the purity of a colony or a culture, inoculate it into peptone water and then streak an EMB plate before trying to screen.

If cultures are still suspicious after being inoculated into dextrose, mannite, and sucrose broth tubes, Gram stain done, and indol test performed, they are put into additional sugars (arabinose, rhamnose, dulcite, xylose and salicin). While these various biochemical reactions are valuable, the final identification depends upon the study of their antigenic structure. If the laboratory is not prepared to do these agglutination tests, the organisms may be placed on a plain agar slant and mailed to a Public Health Laboratory or a typing center.

SUMMARY

Procedures are outlined for the isolation and identification of members of the enteric group of bacteria. Post-cathartic feces and urine specimens are streaked (so there are isolated colonies) onto Hajna-Perry modification of Wilson-Blair, Shigella-Salmonella and Eosin Methylene Blue plates, and inoculated into Selenite. Friewer Shaughnessy medium, with its check on motility, production of acid or acid and gas from lactose, and production of hydrogen sulfide, is used for screening. Organisms surviving thus far are inoculated into sugars, Gram stained, and tested for indol. Tentative differentiation and identification is confirmed by serological reactions.

This method is very useful in laboratories where a large volume of work is done and will also lend itself well to the small laboratory.

The routine is essentially that in use in the State of Illinois, Department of Public Health Laboratories and in the institution laboratories throughout the state that are approved by that Department.

APPENDIX

Hajna Perry (Wilson Blair) Agar

30 gm. Agar
10 gm. Peptone (Bacto)
5 gm. Meat Extract
5 gm. Sodium Chloride
1,000 ml. Distilled Water

Autoclave 20 minutes at 121° C. (15 # pressure).

Adjust the melted base to pH 7.4-7.6 with N sodium hydroxide and add:

10 gm. Dextrose (dissolved in 5 ml. sterile water).
70 ml. Hajna Perry Reagent
5 ml. 1% Aq. Brilliant Green

Pour plates rather thick and dry with lids partially removed. Plates should be made fresh every four days. Store in refrigerator.

Bacto-Wilson Blair Base may be secured in dehydrated form from Difco Laboratories, Inc. If this is used, make plates according to directions on label.

Hajna Perry Reagent (Selective Reagent)
(Must not be older than six weeks)
Dissolve:
60 gm. Anhyd. Sodium Sulfite (Merck) in 200 ml. distilled water.
20.5 gm. Bismuth and Ammonium Citrate (Mallinchrodt) in 150 ml. water.
31.5 gm. Anhyd. Dibasic Sodium Phosphate (Mallinchrodt) in 100 ml. water.
2.4 gm. Anhyd. Ferrous Sulfate (Baker's Dried) in 30 ml. water. Acidify with 2 drops Conc. HCl.
Mix in order given and boil until slate gray. Agitate constantly during boiling.

Bacto-S S Agar is prepared according to directions on Difco label. Store in refrigerator and use within two weeks.

Bacto-E.M.B. Agar is prepared by directions on Difco label. Add 5 gm. agar per 1000 ml. Plates are poured aceptically with the agar at 45° C. Store in refrigerator. Keeps about two weeks.
(Baltimore Biological Laboratories, Inc. also make an EMB Agar.)

Selenite F Enrichment (B.B.L.)
or
Bacto-Selenite Broth (Difco)
Prepare as directed on label. Tube in 10 ml. amounts in 18 x 150 mm. tubes. Store in refrigerator.

Lead Semisolid Agar (Friewer Shaughnessy medium)
5 gm. Agar (Bacto)
30 gm. Peptone (Bacto)
3 gm. Meat Extract
1,000 ml. Distilled Water
Autoclave 15 minutes at 121° C. (15 # pressure).
Place in water bath and cool to 50° C.
Adjust the melted agar to pH 7.8 with N NaOH and add:
10 gm. Lactose
2.5 ml. Brom Thymol Blue (0.4% aqueous solution)
0.5 gm. Lead Acetate (Lead Subacetate Merck) dissolved in 10 ml. distilled water.
Fill sterile 16 x 150 mm. tubes to a depth of about 3 inches. Autoclave 15 minutes at 115-118° C. (10-12 # pressure). Store in refrigerator. Place stoppers over cotton plugs if it is to be kept for some time.
Difco Laboratories, Inc. make Bacto-Friewer Shaughnessy Base No. 1 and Bacto-Friewer Shaughnessy Base No. 2 in dehydrated form which may be used to make this medium. Follow directions on label.

Sugar Base
10 gm. Peptone (Bacto)
5 gm. Sodium Chloride
3 gm. Meat Extract
1,000 ml. Distilled Water
Heat if necessary to dissolve. Adjust pH 7.6-7.8 with N NaOH. Add 5 ml. of 0.4% Aq. Phenol Red. Flask in convenient amounts. Autoclave 20 minutes at 121° C. (15 # pressure).

Sugars
Dextrose } 0.6%
Mannite }
Add 6 gm. sugar to 1,000 ml of Base (1.5 gm. to 250 ml.) Readjust pH 7.6-7.8. Tube using sterile 16 x 150 mm. tubes with Durham vials. Autoclave 15 minutes at 115° C. (10 # pressure).
Sucrose } 1.0%
Lactose }

Add 10 gm. sugar to 1,000 ml. of Base (2.5 gm. to 250 ml.) Readjust pH 7.6-7.8. Tube using sterile 16 x 150 mm. tubes. Place an inverted Durham tube in each one. Autoclave 10 minutes at 115° C. (10 # pressure).

Rare Sugars

These sugars are destroyed by heat and must be added aseptically. Filter through Seitz filter.

Inulin, Dulcite, Dextrin, Salicin, Galactose — 5% aqueous. Warm to dissolve before filtering.

Rhamnose, Cellobiose, Raffinose, Inosite, Maltose, Sorbite — 10% aqueous. Warm to dissolve before filtering.

Arabinose, Xylose — 10% aqueous. DO NOT HEAT.

To make 0.5% concentration:
Add 0.5 ml. of 5% solution per 5 ml. Base,
or 0.25 ml. of 10% solution per 5 ml. Base.

Brom Thymol Blue—0.4% aqueous solution. With mortar and pestle grind thoroughly 1 gm. Brom Thymol Blue. Add approximately 16 ml. (freshly made) 0.1 N NaOH, grinding and decanting as necessary. Make up to 250 ml. with distilled water. Add NaOH dropwise, shaking thoroughly, until the dye is in solution, i.e., approximately 4 ml.

Phenol Red—0.4% aqueous solution. Grind thoroughly 1 gm. Phenol Red in motar. Add 30 ml. of 0.1 N NaOH slowly to make a thick paste. Continue grinding and pause to decant when necessary. After all of NaOH is used, continue same procedure with 220 ml. of distilled water.

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THE CULTIVATION OF VIRUSES: A REVIEW*

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PART III

Virus Cultivation by Animal Inoculation

Experimental animals such as mice, rats, monkeys, dogs, cats, and fowl, have been used with success to determine the existence and, in some cases, the concentration of viruses present in inocula. A great amount of work has been done using various laboratory animals to establish susceptibility to the viruses and to find the route of inoculation best able to demonstrate clinical and pathological evidence of the infectious agent inoculated.

The following shows the host range and the route of inoculation of some of the more common viruses.

Australian X. Susceptibility was found in rhesus monkeys, sheep, a horse, a foal, and a calf. Rhesus monkeys showed signs of lethal meningoencephalitis 5 to 23 days after intracerebral injection. Dogs, kittens, rabbits, guinea pigs, and fowl were found to be insusceptible.

B. virus. Susceptibility has been shown in rabbits, monkeys, and guinea pigs,and, irregularly, in mice, only to an age of three weeks. The rabbit, inoculated by peripheral injection, is the animal of choice. After intravenous injection in monkeys a striking exanthem and enanthem appears about the head region. The disease progresses as an acute ascending myelitis with death resulting.

Bwamba Fever. Mice are susceptible intranasally or intracerebrally. Rhesus monkeys are mildly susceptible. Guinea pigs and rabbits are apparently immune.

Lymphocytic Choriomeningitis. Armstrong and Lillie[67] found that lymphocytic choriomeningitis is transmissible to monkeys. Traub[68] found mice most susceptible when infected by intracerebral and intranasal routes. Susceptibility has also been shown in guinea pigs, dogs, rats, and chimpanzees. Albino mice and guinea pigs, injected intracerebrally, are considered the animals of choice. In mice, tremors and convulsions lead to generalized rigidity with death following within about 9 to 16 days after inoculation.

Common Cold. This is infective for man and chimpanzee. All other animals tested appear to be insusceptible.

Colorado Tick Fever. Hamsters are susceptible intraperitoneally after 12 passages. Mice are killed by intracerebral injections of the virus. Mice more than fourteen days old must be injected with an adapted strain to show clinical symptoms of paralysis and prostration.

Coxsackie Group. Infant mice and hamsters are susceptible and develop weakness and paralysis of one or more extremities and of the neck within 2 to 10 days after inoculation. Infant mice may be infected by intracerebral, intraperitoneal, and subcutaneous routes, and are relatively susceptible by the oral route. Other laboratory animals seem to be immune or else to develop a subclinical infection resulting in a carrier condition.

Dengue. Sub-clinical infections have been produced in monkeys and chimpanzees by intracerebral inoculation. Several strains have been adapted to mice by intracerebral inoculations. Dogs, young hogs, rabbits, guinea pigs, white mice, white rats, hamsters, and cotton rats appear to be insusceptible.

Encephalitis: Eastern, Western, Japanese B, Venezuelan, St. Louis. The mouse is the animal of choice for demonstration of these viruses. Hamsters, rats, guinea pigs, and puppies are also susceptible. All animals are inoculated intracerebrally. The nasal route is successful with mice. Host range is similar for all types of encephalitis with the exception that the eastern variety is more virulent and will infect sheep, cats, hedgehogs, and quail, in addition to the host range of the other types of encephalitis.

Herpes Simplex. Rabbits, rats, hamsters, mice, and guinea pigs are susceptible by corneal, intracerebral, intraperitoneal, or intradermal inoculation. The latter route is satisfactory for dermotropic strains.

Influenza. Ferrets, mice, the European and Syrian hamsters, hedgehogs, and gray rats are susceptible. The intranasal route is used for all inoculations of these animals. Striking clinical symptoms of the disease are obtained only by use of adapted strains of the virus.

Lymphogranuloma venereum. The monkey and the mouse are the animals of choice for this virus. Monkeys have been infected by intraperitoneal, intrapreputial, intracutaneous, intrapulmonary, and intraocular injections. The most common route for mouse inoculation is intracerebral. Results have not been definite with other experimental animals.

Measles. Monkeys are susceptible to measles inoculated by intramuscular, intracerebral, intravenous, intraperitoneal, intradermal, and subcutaneous routes. No other animals have been found susceptible to this virus. Exanthem and enanthem develop, similar to the manifestations of measles in man.

Mumps. Only a few species of monkeys, such as the rhesus, are susceptible to this disease. The chick embryo is used for most of the experimental work on this virus.

Newcastle Disease. Birds, mice, rats, hamsters, and monkeys are susceptible when inoculated intracerebrally. Other routes produce the disease in experimental animals irregularly. Best results are obtained with fowl.

Poliomyelitis. Primates and rodents are susceptible. Direct contact of the virus with nervous tissue is needed, which involves usually either the intracerebral or the intranasal route. The animal of choice is the chimpanzee which is highly receptive and may be infected by feeding.

Psittacosis. Mice are the animals of choice, although birds will develop the disease readily. Mice are inoculated by intraperitoneal, intracerebral, intravenous, or subcutaneous routes, and can also be infected by feeding. Death usually occurs in from 3 to 30 days.

Rabies. Rabies apparently is infectious for all mammals. All routes of inoculation may be used except feeding. However, for diagnostic purposes, rabbits, guinea pigs and special strains of mice are generally used. Infection by the intracerebral route is preferred.

Rubella. This virus will cause mild cases of the disease in monkeys infected by subcutaneous, intraperitoneal, intranasal, or intravenous routes. Other animals seem to be insusceptible.

Smallpox. Many animals are slightly susceptible to this disease, but results are confusing because some of these animals are also susceptible to vaccinia which is often being studied in the same laboratories. Corneal inoculations in rabbits will cause keratitis which has been used as the basis for a diagnostic procedure for variola. However, rabbits are sometimes used to differentiate between the viruses of vaccinia and variola. Serial transfers of the latter have not been demonstrated, whereas vaccinia grows readily in rabbits.

Vaccinia. Rabbits, mice, monkeys, and guinea pigs are perhaps the most frequently used for laboratory inoculation. A number of routes can be used to produce infection with this virus.

Yellow Fever. Monkeys, especially the rhesus, are used to demonstrate the viscerotropic virus of yellow fever, while mice are used for the neurotropic strains. The mice are inoculated intracerebrally for the best demonstration of this virus. Hedgehogs and guinea pigs are also susceptible. Rabbits and rats have been found to be insusceptible.

Virus cultivation by animal inoculation is used extensively to demonstrate virus multiplication after cultivation in tissue cultures and in the embryonated egg, especially in those cases where no typical lesions are produced and other indicators of growth are not applicable.

For general virus cultivation, animal inoculation is perhaps the least desirable of the three general types of cultivation. In many cases laboratory animals are difficult to handle. Freedom from infection before inoculation is difficult to control, especially where the animal quarters used are not kept under optimum conditions. Occasionally animals used for inoculation will have an antibody titre against the virus inoculated because of a previous infection, and most animals will produce antibodies against an inoculated virus. Animals are prone to cross infec-

tion which may cause confusing results when the results of inoculation are evaluated. Finally, animals are expensive to purchase and to maintain.

Time and labor spent in caring for laboratory animals will be amply repaid by reduction in latent infection and handling difficulties, to say nothing of the moral aspect of the problem.

Cultivation of the Bacterial Viruses

The bacteriophage was first reported by Hankin,[69] in 1896, who detected phage activity against vibro cholera in certain rivers in India. Twort,[70] in 1915, isolated a bacteriophage active against a micrococcus. D'Herelle,[71] in 1922, published a monograph on the bacteriophage which included extensive descriptions of the techniques used for the isolation and cultivation of the bacteriophage. Recently, the impetus for the study of the bacteriophage has been increased because it is felt that information obtained on the growth and reproduction of the bacteriophage may provide a clue to the many problems related to the mechanism of growth and reproduction of the plant and animal viruses. The bacterial viruses are studied in preference to the other types of viruses perhaps because of the simplicity of cultivation, coupled with their rapid growth with susceptible bacterial cells.

Isolation procedures vary with the type of phage desired. In general, a phage is most readily isolated from fluids which contain the bacterial host normally. For example, if a phage active against *Escherichia coli* is to be isolated, then sewage would probably be the most productive source of the desired phage. Specimens are taken from the source and are passed through bacteriological filters. Assuming the phage was present in the sample, it is found in the liquid which passed through the filter.

If a phage active against a specific organism is desired, varying amounts of the filtrate are added to tubes of broth previously inoculated with the test organism. One inoculated tube to which no filtrate is added serves as a control. Clearing of the turbidity of the experimental tubes is an indication of phage activity. More than one tube should be used for each attempt at phage isolation so that different amounts of filtrate can be used as an inoculum. If not, a phage of low virulence may escape detection.

On solid media, bacterial viruses will form colonies which appear as plaques on a heavily seeded plate. This phenomenon of plaque formation has been utilized to measure the viable count of an unknown suspension of phage in much the same manner that plate counts are used in bacteriological procedures.

Successful cultivation of a phage depends on a number of factors. A phage can multiply only in a living, susceptible bacterial cell. The medium in which the phage and bacteria are

suspended must sustain the life of the bacteria while phage multiplication takes place, and should not alter the constitution of the bacterial cell. D'Herelle[71] found that the medium used for multiplication of the phage should be slightly alkaline because even slight acidity will cause cessation of phage reproduction. Temperature regulates the velocity of phage reproduction in that the optimum temperature for reproduction is the same as that for reproduction of the host cells. Any significant deviation from this temperature will cause inhibition of phage multiplication.

Because living bacterial cells are necessary for phage reproduction, cultures less than 24 hours old are most desirable.[71, 72] Although actively reproducing cells give higher rates of phage reproduction, this activity is not essential to phage multiplication,[73, 74] because bacterial growth and phage growth apparently are separate functions.[75]

In summation, the cultivation of the bacteriophage is, in itself, a simple process—that of the addition of the phage to living susceptible bacterial cells. However, when one tries to hypothesize the mechanism of growth and reproduction of the bacteriophage, the problem becomes complex.

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THE ACCURACY OF URINE SUGAR TESTS†

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INTRODUCTION

Tests for urine sugar represent one of the most frequently performed clinical chemical tests. Since such tests are all quite simple there is a tendency to consider that they are always correctly performed and always give correct results. The present report is concerned with the accuracy of Benedict's qualitative test[1] and Clinitest.‡ Benedict's qualitative test is a widely used procedure in which a solution containing copper sulfate, sodium citrate and sodium carbonate is heated with a few drops of urine. In the presence of reducing sugar, the color of the solution changes from blue to green and then to yellow or orange. Clinitest is a simplified test for urine sugar in which all the ingredients for a test are contained in a tablet. When a Clinitest tablet is added to a definite quantity of diluted urine, the ingredients react with the liberation of heat and if sugar is present, the blue cupric compounds are reduced to yellow or orange cuprous compounds. Comparison of the reaction mixture with a color chart indicates the approximate sugar concentration of the urine sample.

METHODS

Benedict's Qualitative Test

The reagent has the following composition:

Copper sulfate	17.3 grams
Sodium citrate	173.0 grams
Sodium carbonate (Anhyd.)	100.0 grams
Distilled water to 1000.0 ml.	

All tests were carried out using the following procedure: To 5 ml. of reagent in a test tube (18 mm. diameter) add 0.4 ml. (8 drops) of the urine to be examined. Mix and place in a boiling water bath for exactly five minutes and then remove and allow to cool spontaneously. The procedure of using a bath for heating was originally described by Myers,[2] whereas the original technic of Benedict[1] employed boiling over an open flame for 1-2 minutes. Reduction is indicated by the appearance of a precipitate and the change of color of the contents of the tube from blue to green and then to yellow or orange or red. Results

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‡Clinitest is the registered trademark of Ames Company, Inc., Elkhart, Indiana.

are read after the tubes have cooled. In the present study six degrees of reaction have been used. These are:

Negative	Clear blue or with turbidity so slight that newsprint can be read through the tube.
Trace	Change of color from blue to green and the appearance of sufficient precipitate to make it impossible to read newsprint through the tube.
1+	Yellowish green (green dominant).
2+	Greenish yellow (yellow dominant).
3+	Brown or orange.
4+	Brick red.

Clinitest

The procedure employed for Clinitest was that described in the direction sheet accompanying this product. These directions are as follows:

1. With dropper in upright position place 5 drops of urine in test tube (Clinitest tube 15 mm. x 75 mm.). Add 10 drops of water.

2. Drop 1 tablet into test tube. Watch while reaction takes place. Do not shake test tube during reaction nor for 15 seconds after boiling inside test tube has stopped.

3. After 15 second waiting period shape test tube gently and compare with color scale.

Interpretation of Test

Negative—the fluid will be blue at the end of a waiting period of 15 seconds.

Positive—if the urine contains sugar, the fluid will change color from blue to green to tan and finally through orange to brown, depending on the amount of sugar present. The shade of color is compared with a color chart to obtain the approximate amount of sugar present.

Benedict's Quantitative Test[1]

The reagent has the following composition:

Sodium carbonate (Anhyd.)	100.0 grams
Sodium citrate	200.0 grams
Potassium sulfocyanate	125.0 grams
Copper sulfate	18.0 grams
Potassium ferrocyanide	5.0 ml. of a 5% solution
Distilled water to 1000.0 ml.	

The procedure is as follows:

To 25 ml. of Benedict's quantitative reagent in a 125 ml. Erlenmeyer flask add 10 grams of anhydrous sodium carbonate. Heat the mixture to boiling and add urine dropwise from a burette until the last trace of blue just disappears. The quantity

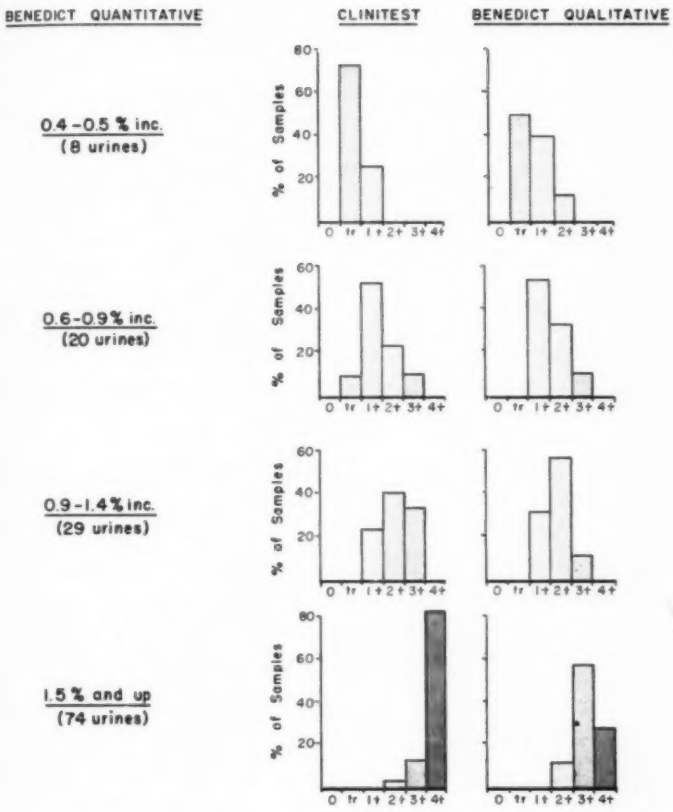

Figure 1. Quantitation of Sugar in Urine. Using Benedict's quantitative results as the criteria, urines have been grouped according to the amount of sugar present. Clinitest and Benedict qualitative values for each group are indicated in the graph.

of urine used contains exactly 0.05 grams of glucose and therefore the percent glucose in the urine may be calculated by dividing five by the milliliters of urine used.

EXPERIMENTAL OBSERVATIONS

Benedict's qualitative test is an extremely sensitive test for urine reducing substances. Several modifications for Benedict's test have been suggested which are designed to reduce the sensitivity. In other instances the physician or the medical technologist has come to recognize that trace or faint reactions with Benedict's test have no significance and they are accordingly disregarded. In the design of Clinitest, one of the objectives was to provide a test for urine sugar that was less sensitive than Benedict's qualitative test. That this has been accomplished can be shown easily by adding extremely small quantities of glucose to normal urine. For instance, the addition of 0.05% (50 mg. per 100 ml.) glucose to most normal urines will result in specimens which give clear-cut trace reactions with Benedict's test, but negative reactions with Clinitest. This decreased sensitivity of Clinitest is a definite advantage of this test over Benedict's qualitative test as will be shown below.

Sugar tests were run on a series of 690 urines from hospital patients. Results obtained with Clinitest and with Benedict's qualitative test are shown in Table I. After testing 551 consecutive hospital urines, the data on positive urines was extended by collecting and studying only those urines which contained sugar. Of the urines studied, 391 were negative with both Clinitest and Benedict's qualitative test, 195 were positive with both tests, and 104 urines were negative with Clinitest but positive (trace only) with the more sensitive Benedict's qualitative test. Every urine that was negative with Benedict's test was negative with Clinitest and every urine that was positive with Clinitest was also positive with Benedict's test. No follow-up study was made on patients whose urines were included in this series.

Table I

URINE SUGAR TESTS ON HOSPITAL PATIENTS

Number of Urines	Clinitest Results	Benedict Results
391	Negative	Negative
104	Negative	Trace
195	Positive	Positive

All urines which contained over ¼% sugar with Clinitest were also tested with Benedict's quantitative reagent. In Figure I results with Clinitest and qualitative Benedict's tests are com-

pared with the sugar values obtained by the quantitative Benedict method. It can be seen from Figure I that results with Clinitest compare favorably with values obtained by the Benedict quantitative method. The results with Benedict's qualitative method do not give as good agreement with the quantitative procedure in the range of higher sugar concentrations. Several urines were observed that contained extremely high concentrations of sugar that gave only a 3+ reaction with Benedict's solution. In our experience the difference between a 3+ reaction and a 4+ reaction with Benedict's qualitative test does not depend on the amount of sugar present but depends on other constituents of the urine which influence the colloidal characteristics of the precipitated cuprous oxide.

Clinitest and Benedict's qualitative tests have also been run on urines from healthy subjects. These urines were collected at various times during the day. They include postprandial specimens as well as urines voided after a short fasting period and those voided after an overnight fasting period. The series includes both dilute and concentrated urines. Table II shows that 304 of the urines were negative with both Clinitest and Benedict's test, that 16 urines gave trace reactions with both tests, and that 57 urines or approximately 15 percent gave trace reactions with the more sensitive Benedict's test while testing negative with Clinitest.

Table II

URINE SUGAR TESTS ON HEALTHY SUBJECTS

Number of Urines	Clinitest Results	Benedict Results
304	Negative	Negative
57	Negative	Trace
16	Trace	Trace

Further studies with healthy subjects have been carried out in order to learn whether trace reactions with Benedict's qualitative test have any significance. One approach has been to obtain additional urine samples from 12 of the subjects whose urines gave trace reactions with Benedict's qualitative test but negative tests with Clinitest on the first test. With the second urine sample from these 12 subjects, all were completely negative with both Benedict's and with Clinitest.

A second approach has been to collect further urines from healthy subjects who had negative tests with both Benedict's qualitative test and with Clinitest on the first sample. In these studies an effort was made to create conditions which might lead to the presence of small quantities of reducing substances in the urine. This was done by collecting concentrated urine samples after mild fluid intake restrictions or urine samples

following a test breakfast of whole wheat toast, jam and coffee. Eight out of 12 subjects who initially had a urine that was negative with both Benedict's test and with Clinitest had at least one urine sample that showed a trace reaction with Benedict's test following this regime. Accordingly, it appears that trace reactions with Benedict's qualitative test have no clinical significance since practically every one at one time or another excretes sufficient reducing substances in their urine to give a trace reaction with Benedict's qualitative test. The decreased sensitivity of Clinitest gives it a definite advantage as a test for the detection of abnormalities of carbohydrate metabolism.

Since Benedict's test is the more sensitive, it would be expected that a urine which gave a negative Benedict's test should also give a negative Clinitest reaction. If any urine which reacted negatively with Benedict's test did not give such a result, it would indicate that one or the other test was in error. Of all urines studied, a total of 695 have been negative with Benedict's test, and every one of these also gave a negative Clinitest reaction. This means that in this series each test gave a correct result. Clinitest is the less sensitive of the two tests and it would be expected that a urine which gave a positive test with Clinitest should also give a positive test with Benedict's test. Here again, if any urine which reacted positively with Clinitest did not give such a result with Benedict's, it would indicate that one or the other test was in error. Of all the urines studied a total of 211 have been positive with Clinitest and every one of these gave a positive reaction with Benedict's test. These results indicate that both Benedict's qualitative test and Clinitest are accurate for the amounts of sugar they are designed to detect.

A relatively large number of non-glucose substances or their metabolites have been reported to give a positive Benedict's test in urine. These are shown in Table III. In most instances the quantity of these substances which occurs in the urine gives only trace reactions with Benedict's test. Since Clinitest is less

Table III

NON-GLUCOSE SUBSTANCES REPORTED TO GIVE POSITIVE BENEDICT'S TEST IN URINE

Ascorbic Acid	Maltose
Caronamide	Nucleoprotein
Chloral	Para-aminosalicylic Acid
Conjugated Glucuronates	Penicillin
Formaldehyde	Ribose
Fructose	Salicylates
Galactose	Streptomycin
Glucuronic Acid	Uric Acid
Homogentistic Acid	Uronates (following hyaluronidase)
Isoniazid	
Lactose	Xylose

sensitive, it will be less likely to give positive results with the various non-glucose substances in urine that are indicated in Table III. This has been established in many instances either with regard to the material in question or with regard to its metabolites.

Table IV indicates possible sources of error in urine sugar testing which may be encountered with the use of Benedict's test and with Clinitest. As we have indicated, almost every normal healthy person may, under some circumstances, excrete trace quantities of reducing substances in his urine. We feel that these minute amounts of reducing substance are normal and only tend to confuse the recognition of abnormalities of carbohydrate metabolism. Excessive heating of Benedict's test will give erroneous results. This is more likely to happen when heating is carried out over an open flame. Inadequate heating of Benedict's test may be a cause for overlooking a glycosuria, as has been pointed out many times. Usually in these cases, the person performing the test is in a hurry and when, after heating the tube in the flame for a few seconds, no color change is observed, it is erroneously reported that the test is negative. If many tubes are placed in a bath, they may sufficiently cool the bath so that they never do get adequately heated. The use of an excess of urine will result in a positive reaction with Benedict's test. Here again, the tendency to save time may result in an excess of urine being employed with resultant errors. Benedict's test by reason of its extreme sensitivity may give false positive results with interfering substances. These most frequently are mere trace reactions. Finally, we have observed that the sensitivity of Benedict's test is further increased by carrying out the reaction in a narrow tube. Many urines which will give a negative test in a tube 18 mm. in diameter will give a trace reaction in a tube 15 mm. in diameter.

Table IV

SOURCES OF ERROR IN URINE SUGAR TESTS

Benedict's Test	Clinitest
1. Extreme sensitivity.	1. Exposure of tablets to moisture.
2. Excessive heating.	2. Failure to use proper amount of urine or water.
3. Inadequate heating.	3. False positives from greater amounts of interfering substances.
4. Use of excess urine.	
5. False positives from interfering substances.	
6. False positives from heating in narrow tubes.	

Table IV also shows sources of error with Clinitest. Clinitest tablets are quite hygroscopic and if they are not kept in a tightly closed container they will pick up moisture from the air. Deterioration of this type is indicated by the development

of an increased blue color and by a roughening of the surface of the tablet. Clinitest tablets which have picked up excess moisture do not give a satisfactory test for urine sugar. Failure to use the requisite number of drops of urine or of water in a Clinitest reaction will lead to errors. The presence of large quantities of interfering substances in the urine will lead to false positive reactions with Clinitest. Examination of several interfering substances indicated that from 3 to 10 times as great a concentration is required to give a positive reaction with Clinitest as with Benedict's test.

Consideration of the factors mentioned in Table IV suggests that errors may occur with each test but that there are fewer sources of error with Clinitest.

SUMMARY

A series of urine samples from hospital patients and from healthy subjects has been tested with Benedict's qualitative test with Clinitest. Benedict's qualitative test is the more sensitive of the two tests for urine sugar, but this appears to be a disadvantage because many urine samples from healthy subjects give trace reactions with this test. In urines which contain sugar, Clinitest measurement of the quantity of sugar gives a good correlation with the amount of sugar determined with Benedict's quantitative method. Both Clinitest and Benedict's qualitative test are completely accurate when properly performed. Mistakes may be made with both methods but are less likely with Clinitest.

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BLOOD VOLUME STUDIES WITH RADIO ACTIVE ISOTOPES

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It has been known for sometime that the blood volume of an individual decreases or increases in various diseases, and following certain medications. A decrease in blood volume is found in cases of hemorrhage, burns, dehydration, shock, and in pernicious and chronic anemias. An increase has been noted in polycythemia vera, cirrhosis of the liver, leukemia, splenomegly with anemia, and in hyperthyrodism. Steriods, cortisone, and ACTH are medications that alter the blood volume.

Although of diagnostic value, blood volume determinations are seldom ordered by the physician for want of a satisfactory procedure. The physician has had to rely on the red cell count and hematocrit for his information. While of help these procedures often give him false information as to the body's total blood volume.

Two semi-satisfactory procedures have been used throughout the past few years. These procedures would probably have continued as the only satisfactory methods for a few more years if it had not been for the discovery of the use of radioactivity for scientific purposes. Therefore two methods of determination are now possible; one determination without use of radioactivity, the other using radioactivity.

The procedures without the use of radioactivity will be discussed only briefly. Two indirect procedures may be used, one one using carbon monoxide, the other a dye procedure.

1. Carbon Monoxide procedure:

A sample of blood is withdrawn anaerobically from the subject and analyzed for its oxygen capacity. The subject rebreathes for 15-20 minutes a known amount of CO (110-150 ml.). Just before the end of the rebreathing period withdraw another sample of blood and analyze for CO. Results are calculated by a given formula.

The disadvantages of this method are: 1) it is not known how rapidly the CO passes from the blood to the tissues. 2) The technic is difficult, especially in very ill patients.

2. Dye procedure:

Evans, Blue dye, T-1824 (E. K. 3873) is used for this procedure in which the results are reasonably good.

Ten ml. of dye are injected intravenously over a period of 30-40 seconds. At ten minute intervals over a period of about 50 minutes, 7-8 ml. of blood are collected. These samples are allowed to clot in waxed, stoppered centrifuge tubes, then centrifuged for 30 minutes, and the serum pipetted off. The specimens of serum are read in the photometer.

This method has several advantages over the previous procedure: 1) the dye is non-toxic, 2) it is non-hemolytic, 3) it is eliminated slowly from the blood stream, 4) it is found only in the plasma and retains its color on entering the blood stream.

The disadvantages of this technic are: 1) it is tedious, 2) requires specially prepared equipment, 3) it must be completed in a few hours and cannot be repeated often.

These are the only two accepted methods for determining blood volume without radioactive material. Several methods have been developed utilizing radio activity.

However before beginning the discussion of the various procedures, it might be well to define exactly what an isotope is, what is meant by radioactivity, and what does the half life of an isotope mean.

Isotopes are atoms with identical chemical properties but different atomic weights. The term includes both the stable and radioactive nuclei of a particular element.[8]

A radioactive isotope is an atom or isotope that has been bombarded with "atomic" particles and its energy level raised, it is now radio active.

Its half life is a measure of the length of usefulness of that radioactive isotope.[8] In order for a certain isotope to be useful in medicine the half life has to be less than 30 days, but still high enough that it can be used over a reasonable period of time.

Isotopes like all material possess certain properties that make them good for some determinations and no good for others. Some isotopes can be used only for determining plasma volume, others for determining the whole blood volume. Iodine 131 or Sodium 24 may be used for plasma volume determinations, Phosphorus 32, Iron 59, and Chrome 51 are used for whole blood determinations.

Iodine 131 is used in the form of tagged human serum albumin, following this procedure:[3]

An amount of tagged albumin is diluted to 20 cc with saline. A small amount is saved for dilution with serum to serve as a standard. The remainder is given intravenously and a sample withdrawn from the opposite area 10-30 minutes later. The sample is centrifuged for 15-30 minutes and the activity of 1-2 cc of the plasma determined by a Geiger or scintillation counter. From this value and the hematocrit, the blood volume is easily calculated.

$$\frac{\text{Ct. Std.} \times \text{Vol. injected} \times \text{Dil. of Std.}}{\text{Cts. in sample cc}} = \text{Plasma Volume}$$

$$\frac{\text{Plasma Volume}}{\text{100-Hematocrit}} = \text{Blood Volume}$$

This is an accurate, simple, and very convenient method. The material is already made up and ready to be injected, it may be stored for a short time as it has a half life of 8 days. The Iodine goes to the protein of the plasma and does not stay in the blood stream for a long period of time. Because of this, the measurement of the blood volume may be made within an hour or two of the initial injection. At the end of one hour it has been found that 90% of the injected protein is still present, at the end of 4 hours, 78% is still present.

The disadvantage of this method is that it may not be repeated immediately, one must wait for a period of days before repeating, similar to the Evans Blue dye method.

Na 24

Na 24 has a half life of only 14.9 hours. The procedure is as follows: Intravenously inject 10 to 20 micro curies of Na 24 in the form of sodium chloride, the material can be dissolved in about 5 cc of distilled water. Ten to thirty minutes later a sample is withdrawn from the opposite area and centrifuged for 15-30 minutes. The activity of 1-2 cc of the plasma is then determined by a Geiger or scintillation counter. From this value and the hematocrit, the blood volume is easily calculated.

$$\frac{\text{Ct. Std.} \times \text{Vol. injected} \times \text{Dil. of Std.}}{\text{Cts. in sample cc}} = \text{Plasma Volume}$$

$$\frac{\text{Plasma-Volume}}{\text{100-Hematocrit}} = \text{Blood Volume}$$

The results of the Na 24 test are comparable with that of Iodine 131. It has the advantages of being easier to count and can be repeated oftener and sooner. However since the period of half life is so short it makes it difficult to work with. Also the material cannot be tagged and kept on hand as can the Iodine 131. Therefore for plasma determinations the Iodine 131 appears to be the method of choice for more accurate results.

As stated before Fe 59, P 32, and Cr 51 are used for whole blood determinations.

Fe 59

Only minute amounts of Fe 59 are used as it has a half life of 45 days and stays in the body. It is not used routinely as a clinical tool although it has been used on occasions. On these occasions a small amount of radioactive iron salt was given intravenously to a blood donor. A sample of blood is withdrawn and transfused to the patient, from which in turn a sample of blood is withdrawn and counted for radio activity.

This test certainly has more disadvantages than advantages.

There is a limitation as to the amount of radio active Fe that may be injected. That which is injected is twice diluted therefore it is too dilute to do an accurate count. The count may be done but is very difficult at the level of activity with which you are working.

P 32

P 32 is easy to use for blood volume determinations as you measure the tagged red cell itself.

Fifteen cc of blood are drawn into a heparinized syringe, and 5 cc are placed in a sterile, rubber capped, centrifuge tube, and incubated at 37° with constant rotation, with ½ mc. of radioactive isotonic sodium dihydrogen phosphate pH of 7.3. At the end of two hours the red cells are washed three times with saline solution and resuspended in the patients' own plasma. One cc of this blood is then injected into the patient, and using the same needle and syringe, 1 cc is placed in a 2000 cc flask. At the end of 15 minutes, 5 cc of blood is withdrawn from the opposite arm into a dry oxolate tube. The blood samples and the standard are then counted in the following manner: 0.1 cc is placed on a small piece of filter paper or an aluminum foil, then dried, wrapped in cellophane and placed around a Geiger-Muller tube. The total red cell volume is then calculated

$$\text{TRCV} = \frac{\text{cpm/0.1cc Std.} \times 2000 \times \text{Hematocrit}}{\text{cpm/0.1 cc blood}}$$

$$\text{BV} = \frac{\text{TRCV}}{\text{Hematocrit}}$$

The plasma volume is obtained from the difference of the blood volume minus the total red cell volume.[2]

The advantages of this method are 1) The radiation is easy to measure, therefore small amounts can be used, 2) it gives an accurate measure of the total red blood cell volume, 3) the entire procedure is simple and, 4) P 32 has a half life of 14 days which makes it easy to work with.

The disadvantages are few 1) the length of time of measurement is small, about 6% of the total P 32 is loss per hour, 2) the red blood cells must be handled with care, not exposed to any impurities, or incubated too long, 3) the stock solution must be kept sterile and free from particles (such as dust) in order to prevent error in estimating the total blood volume.

Cr 51

Cr 51 with a half life of 26 days is the newest isotope to be added to the growing list of radioactive isotopes for blood vol-

ume determinations. We have been doing research with this isotope for almost a year now and have found it very easy to work with. For this procedure a neutral solution of sodium chromate (Na2CrO4) is added to plasma free O RH Negative blood.

Three prerequisites are necessary for the use of Cr 51, 1) the Cr 51 has to be in the form of Na2CrO4. Cr 51 as received from Oak Ridge is in the form of Chromic Chloride $CrCl_3$, a trivalent ion which will stay in the plasma and not go through the stroma of the red cell. The results would be the same as with 1131. As Na2CrO4, Cr 51 is a hexa-valent ion which actually permeates the stroma of the red blood cell into the hemoglobin. 2) A neutral solution of Na2CrO4 has been used. If the solution is too basic it will hemolize the cells. 3) The Na2CrO4 has to be added to plasma free cells. On our first experiments we were finding more activity in the plasma than in the red cells. To correct this three methods of treating and handling the cells were tried using radio active blood. 1) Washed cells were resuspended in NaCl. 2) Plasma free unwashed cells were suspended in NaCl. 3) Unwashed whole blood. All three tubes were handled in three different ways, they were 1) Incubated ½ hour at 37°, 2) they were shaken at room temperature for two hours., and 3) they were stored overnight at 7° C. From this we concluded that the best results were obtained with unwashed, plasma free cells suspended in NaCl and stored overnight at 7° C. (Table 1.)

TABLE 1

Methods	Uptake
Washed cells in saline Shaken at R. T. for 2 hours	90.5%
Washed cells in saline Incubated at 37° C for 30 minutes	67.0%
Washed cells in saline Overnight at 7° C	93.0%
Whole blood Shaken for 2 hours at R. T.	67.5%
Unwashed plasma free cells in saline Shaken for 2 hours at R. T.	87.7%
Unwashed plasma free cells in saline Overnight at 7° C	99.0%
Unwashed plasma free cells in saline Incubated at 37° C for 30 minutes	82.0%

From this we concluded that the best results were obtained with unwashed, plasma free cells suspended in NaCl and stored overnight at 7° C.

As the result of this data we have developed the following procedure (asceptic technic is used throughout).

1. Centrifuge 250 or 500 cc of fresh O Rh Negative bank blood in cold centrifuge cups for 20-30 minutes.
2. Remove the plasma and save.

3. Add the neutral solution of Na2CrO4 (number of cc depending on the millicuries of Cr present).
4. Add sterile cold isotonic saline to original volume, mix well.
5. Place the bottle on its side in the refrigerator for 24 hours with frequent rotation.
6. The next day remove the saline-Na2CrO4 solution and save.
7. Wash the cells twice with cold isotonic saline, saving each wash.
8. After the last wash add the plasma removed the previous day.
9. Count by means of a scintillation counter the amount of activity present in the decant, washes and cells. Uptake is usually approximately 95%.
10. Have 20-50, sterile 15-25 cc bottles on hand. To each of these add 12 cc of the radioactive blood.
11. Counts are made on each sample.

The blood is now ready for injection. It may be used up to 21 days (the usual life of bank blood). For each patient (if done on different days) use 2 samples of blood. Inject one sample and keep the other for a control.

1. Inject 10 cc of the radioactive blood into the arm vein of the patient.
2. Rinse the syringe and needle with water, adding the rinse water to the bottle from which the blood was removed.
3. Thirty minutes later withdraw 10 cc of blood. (It is assumed it is evenly mixed at this time.)
4. Count all three samples (standard, rinse and withdrawn sample) again using the scintillation counter.
5. The blood volume is a ratio of the two.

$$\text{Blood Volume} = \frac{\text{Counting rate of the standard}}{\text{Counting rate of the patient}}$$

The blood volume of the patient may be determined for a period of several days. A minimum of three days and a maximum of 6-7 days.

We have found this method and results to be satisfactory although there is still a great deal of work to be done. The outstanding advantage of this method is the ease of preparation of the radioactive material, its long storage time and long half life, whereby it stays in the stroma longer and you are able to repeat the measurement without introducing more activity. A patient's blood volume may be determined at any time the physician desires it, it may be done immediately, a few hours or days later, or followed over a period of time. There is no waiting for material, it is always there, there is no need to rush as the activity will remain for a reasonable period of time, and most important

there is no danger of over radiating the patient. We believe that in a short time this will be the method of choice.

However, since no method is perfect, this procedure also has one disadvantage, the radiation is of a very low energy making it difficult to measure, but we hope to conquer this difficulty soon with improved counting technique.

In conclusion we can say that there is an increasing interest in blood volume determinations, and that the radioactive approach appears to offer the simplest and most reliable method.

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THE FRACTIONATION OF HUMAN BLOOD CELLS AND PLASMA AND ITS APPLICATION IN COAGULATION STUDIES*

By GEORGE Y. SHINOWARA Ph.D.**

Recent reviews on the subject of blood coagulation list as many as 17 plasma or serum factors and 4 platelet factors. These numbers reflect the extensive and productive work being done by numerous investigators throughout the world. On the other hand, theses numbers are also indicative of the complexities of this subject not only to "coagulationists" but also to other scientific workers. Undoubtedly, some of these factors will be proven to be similar or identical by future immunological, chemical and physical characterizations. The inter-reactions of so many currently reported factors can account for the numerous modern concepts of the coagulation mechanism.

Partially in view of this situation, the Department of Pathology undertook an intensive and definitive investigation of this subject. Since some of the experimental principles employed are unique, your Society has invited me to present the observations recorded thus far. Despite my sincere reluctance to review our own work, I am appreciative of this opportunity to acknowledge the technical assistance of members of your Society in this work. Coagulation is a subject embracing many scientific fields such as hematology, biochemistry, physical chemistry, physiology and pathology. Therefore, the medical technologist with her diverse but well organized training is particularly qualified to become a contributor in the advancement of blood coagulation knowledge.

EXPERIMENTAL PRINCIPLES

Isolation of Coagulation Components: Fractions of blood plasma and cells are obtained from clinically feasible volume of sample by physical means resulting in the separation of coagulation factors. Thus, only one known variable is investigated at one time. The products obtained are also in the native state.

Biological Reagents of Human Source and Known Strength: The reagents are biologically pure; i.e., each has only one known coagulation factor. They are obtained by large scale fractionation of human blood and are stored in a lyophilized state. These stable reagents are identified as to concentration in units.

Standards and Controls: With the exception of fibrinogen, the concentrations of the biological coagulation factors are referable to National Institutes of Health human thrombin. In substrate-enzyme reactions, all the components are tested independently

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as controls. Where possible in clinical studies, normal, hypocoagulative, and hypercoagulative blood specimens are tested in mixed series.

PROCEDURES

Collection of Blood: Approximately 15 ml. of blood are obtained by venipuncture with a syringe containing 1.5 ml. of 4 per cent sodium citrate solution, pH 7.3. A definite volume of the citrated blood is removed for the determination of final phase coagulation time. The remainder is centrifuged in calibrated 15 ml. graduated tubes at 1° C., 1,650 xg for thirty minutes. The cell and total volumes are recorded: The citrate dilution factor is obtained from these data. The cells and plasma are processed immediately or stored at —25° C.[8]

Reagents: Details for the preparation and standardization of the reagents are given in articles concerning each quantitative determination. However, subsequent to these publications, certain technical improvements have been made so that prothrombin, fibrinogen, thrombin, thromboplastic plasma component and thromboplastic cell component reagents, all prepared from human blood, are stored in a dry state under vacuum and at —25° C. Since the weight and unit activity are known, it is only necessary to reconstitute the powders with buffer for the preparation of most reagents. These reagent solutions are stable for at least a working day.

Final Phase Coagulation Time: To 0.8 ml. of whole citrated blood is added 0.2 ml. of standard human thrombin 5 U. per ml., and the clotting time is determined. Another clotting time is determined by the same technic except that the standard thrombin reagent also contains 0.2 mg. protamine sulfate per ml. In seventy-five normal individuals the average clotting time with thrombin was 16.9 seconds with a range of 13.9 to 22.0. The clotting time with protamine-thrombin reagent averaged 0.7 of a second faster. This is a rapid screening procedure for the presumptive detection of hypofibrinogenemia and heparinemia.[14]

Fractionation of Plasma: This is accomplished by the application of the low temperature-alcohol principle to a small volume of plasma. After the removal of Fraction I, the procedure differs from Method 6 of Cohn et al[1] since it is specifically designed to separate the plasma coagulation factors. Thus Fraction I sediment obtained from 4 ml. of plasma by low temperature alcohol precipitation is dissolved in 4 ml. citrate-phosphate buffer.[11] The alcohol-plasma supernatant is diluted and the prothrombin Fraction II is precipitated at pH 5.05 and reconstituted to 4 ml. with buffer.[8] The details of the further separation of the supernatant decanted from Fraction II resulting in Fraction III containing antithrombin globulin and Fraction IV which is principally albumin will be published in the near future.

Fibrinogen: The fibrinogen concentration is determined as thrombin-clottable protein on the Fraction I solution by the chemical technic.[11]

Thrombin: The stop watch with a hidden face is started at the moment 0.2 ml. of thrombin solution or activation mixture is blown into 0.8 ml. standard fibrinogen solution, contained in a test tube (i.d., 1 cm.) previously equilibrated in a 37.5° C. water bath for 20 seconds. During the first 4-second period, the pipette is put aside and the test tube is shaken. Then the tube is placed in an almost horizontal position, lowered to a 25° angle and restored to the horizontal position at the rate of 3 cycles every 5 seconds. The first visible clot formation is taken as the end point. The unit of thrombin 0.2 ml. is found by referring to a clotting time concentration curve obtained by repeated tests with N. I. H. thrombin solutions. This reference curve should be prepared by each laboratory.[8]

Thromboplastic Plasma Component (TPC): Two ml. of Fraction I solution are heated in a water bath at 56° C. for 5 minutes. The resulting fibrin is removed by centrifugation in a Lusteroid tube at 32,000 xg, 1° C. for 30 minutes. The supernatant solution, Fraction I-B, is tested as follows: To varying dilutions of Fraction I-B are added known concentrations of prothrombin and thromboplastic cell component reagents. The addition of calcium begins the activation period. Thrombin is measured as previously described at the end of thirty minutes activation at 25-28° C.[14]

Prothrombin: The activity of Fraction II is determined by the homologous isolation procedure[8] which is identical to that described previously for TPC except that the constant or the reagent is placental tissue thromboplastin.

Thromboplastic Cell Components (TCC): Approximately 5 ml. of whole blood cells are homogenized and centrifuged for 30 minutes at 32,000 xg. (Subsequent to the publication of the original technic, the relative centrifugal force and centrifugation time have been increased to 53,000 xg and 1 hour respectively.***) The resulting sediment is titred for TCC activity:[14] The constants or reagents are prothrombin and TPC.

DEFINITIONS: ONE UNIT OF COAGULATION FACTORS

Thrombin: "that in 0.2 ml. volume which will clot 0.8 ml. of fibrinogen solution containing 0.15 to 0.30 percent clottable protein in 15 seconds $\Gamma/2$, 0.129; pH 7.2."[8]

Tissue Thromboplastin: "minimum required to convert one unit prothrombin to one thrombin unit."[8]

Prothrombin: "that which when fully activated at pH 7.2, 25

*** G. Y. Shinowara, "Chemical and Physical Characteristics of the Coagulation Component in Human Blood Cells" presented at the Second Annual Symposium of Blood, Jan. 17, 1953, Detroit.

to 28° C, with optimum concentration of calcium and known excess thromboplastin (or TPC plus TCC), will result in one unit of thrombin within 30 minutes."[9]

Thromboplastic Plasma Component (TPC): "minimum required in the presence of a known excess of TCC and with optimum calcium concentration to convert one unit prothrombin into one unit thrombin within 30 minutes at pH 7.2, at 25 to 28° C."[14]

Thromboplastic Cell Component (TCC): "minimum required in the presence of a known excess TPC and with optimum calcium concentration to convert one unit prothrombin into one unit thrombin within 30 minutes, at 25 to 28° C."[14]

IN VITRO RESULTS

Thrombin-Fibrinogen Reaction: With the exceptions of fibrinogen and calcium, the quantitative measurement of all coagulation factors is based on the rate of fibrin formation. Therefore, the thrombin-fibrinogen reaction was investigated. That an optimum concentration of the substrate results in the greatest velocity of this reaction was confirmed.[5] It was shown that plasma albumin further increased and globulins, in general, decreased the reaction rate.[6] From these findings, it was concluded that coagulation reactions could be most simply studied in low protein systems. The technic for thrombin titration employing a standard solution with optimum fibrinogen concentration was described.[8] With Dr. L. Rosenfeld, the reaction kinetics of thrombin and fibrinogen solution of less than 0.025% concentrations (below optimum) were investigated.[11] Such a system could be expressed by the equation: $K = 1/ct^2$, where ct refers to clotting time in seconds. Very low concentrations of heparin have variable effects on the reaction of thrombin and fibrinogen in Fraction I.[7]

Prothrombin: The preparation from plasma of a stable thrombin- and fibrinogen-free prothrombin fraction was reported.[8] The absence of anti-thrombin and anti-thromboplastin substances in this fraction was demonstrated by reactions with placental thromboplastin. Moreover, this reaction was not accelerated by the addition of plasma or its fractions. These findings resulted in the homologous isolation technic for prothrombin concentration.

Thromboplastic Cell Component (TCC) and Thromboplastic Plasma Component (TPC): "Repeated attempts had been unsuccessful in this laboratory to increase the extent and the rate of the tissue thromboplastin activation of prothrombin with plasma and its derivatives. In this conjunction Fraction I particularly has been tested in such experiments because of its established effectiveness in lowering the gross clotting time when injected into hemophiliacs. The plasma prothrombin and placental thromboplastin lipoprotein employed in these experi-

ments were of human origin and had been quantitatively standardized. Equally unsuccessful were attempts to isolate from blood cells and plasma by high gravity centrifugation a substance with the biological activity of tissue thromboplastin. However, in the high gravity sediment of blood cells was found a lipoprotein which not only clarified but also reconciled the negative results in the two apparently divergent experiments designed to further increase the prothrombin-tissue thromboplastin reaction and to isolate thromboplastin from blood. This blood cell lipoprotein and the nonclottable globulin of Fraction I, together but not separately, were found to be identical to tissue thromboplastin in respect to prothrombin activation."[14] This lipoprotein of whole blood cells and the nonclottable globulin of Fraction I have been termed thromboplastic cell component (TCC) and thromboplastic plasma component (TPC), respectively. The experimental data demonstrate a stoichiometric, rather than a kinasezymogen, relation between these two factors. TPC is identical to antihemophilic globulin reported by Taylor et al[2] in the nonclottable proteins of Fraction I. Proteolytic enzyme activity has been demonstrated without chloroform or kinase activation in this fraction.[4]

THE COAGULATION MECHANISM

The reaction of the biochemical factors, all isolated from human blood and not involving tissue thromboplastin, are as follows:

$$\text{Prothrombin} + \underset{\text{Thromboplastic Plasma Component}}{\overset{\text{Thromboplastic Cell Component}}{}} + \text{Ca} \rightarrow \text{Thrombin}$$

$$\text{Fibrinogen} + \text{Thrombin} \rightarrow \text{Fibrin}$$

The foregoing is not intended to be a theory: It is a concept based on the available direct evidence. Despite its simplicity, it can be the basis for the understanding of certain processes. Normal blood remains fluid as long as TCC is not released by the blood cells. In pathological states, hypo- or hyper-coagulability of blood could be caused by a deficiency or excess of any of these factors. This coagulation mechanism[14] is a framework to which other factors can be added when adequately identified. (If there is tissue injury with the release of thromboplastin, prothrombin can be activated by both this substance and TCC plus TPC.)

STUDIES ON TRANSFUSION WHOLE BLOOD AND PLASMA

Effect of Storage: In whole blood stored at 3-5° C for 28 days, fibrinogen and prothrombin were found to be stable, whereas the TPC concentration dropped 50% in 24 hours.[19] In liquid plasma stored 6 months at 3-5° C, fibrinogen was stable[12] but the prothrombin concentration was down about 50%.[9] TPC, prothrombin and fibrinogen are stable in both frozen and dried plasma for years.[9, 12, 18]

Effect of Ultraviolet Irradiation: The process of ultraviolet irradiation or the irradiation, per se, can destroy almost completely TPC activity in normal human plasma.[18] Prothrombin and fibrinogen are not affected.

CLINICAL RESULTS

Almost 1500 blood specimens from patients with a variety of pathological conditions have been investigated to date. Thus the few observations that have been recorded will be briefly presented.

Normal Values: The coagulation factors and normal values are: fibrinogen, 0.26, S. D. ±0.05%[11]; prothrombin, 81.8 S. D. ±14.4 units/ml.[10]; TPC, 60.4, S. D. ±34.8 units/ml.[20]; and TCC, 52,000, range 17,000-133,000, units/ml. whole blood cells (see footnote, p. 300). The tremendous excess of TCC in normal blood cells can be stated as follows: The release of only 0.1% of the total TCC lipoprotein is sufficient together with TPC to convert all the prothrombin in a unit volume of normal blood to thrombin. Stated in another way, there is in 1 ml. whole blood enough TCC to potentially result in thrombin potency that can clot 5 gallons of standard fibrinogen solution in 15 seconds.

Effect of Dicumarol: Together with Dr. W. B. Smith, prothrombin levels in patients receiving dicumarol were investigated.[20] Discrepancies between the one stage and isolation prothrombin values were noted only during first week of therapy.

Fibrinogen Levels in Various Pathological States: Analysis of almost 1000 specimens disclosed great elevations in fibrinogen in a variety of conditions characterized by inflammatory or necrotic processes: However, it was low in viral infections.[16, 17] The beta naphthol reaction with plasma was found by Drs. Smith and Rosenfeld[13] to be related to the concentration of fibrinogen. This finding is contrary to that of Cummine and Lyons[2] who concluded that qualitative changes in fibrinogen were responsible for fibrin formation by beta naphthol.

Acute Pancreatitis: Together with Drs. Waite and Saleeby, the plasma coagulation factors in acute pancreatitis were determined.[20] In patients, the TPC values were 5 to 22 times the normal mean several days following the serum amylase peak. This observation was confirmed in dogs with sublethal attacks of acute pancreatitis, experimentally induced by tying off the pancreatic ducts.

Hemophilia: Prothrombin, fibrinogen, TPC, and TCC were determined in 12 known hemophiliacs: TPC was practically absent in all of these individuals.[15] This observation has been confirmed repeatedly subsequent to the original report.

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LABORATORY AIDS IN THE DIAGNOSIS OF VINCENT'S INFECTION*

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The use of smears as an aid to the clinician in the diagnosis of necrotizing ulcerative gingivitis, or Vincent's infection, is customary. Usually the preparation of these smears is the duty of the Medical Technologist, and it will be worthwhile to consider the problems involved and the importance of standardizing all phases of the procedure.

Note here that the term necrotizing ulcerative gingivitis was officially adopted by the Committee on Nomenclature of the American Academy of Periodontology in 1947 in place of the misnomers, "Trenchmouth," Vincent's stomatitis, Vincent's angina, or any of the other synonyms previously applied. However, for the sake of clarity in this paper, it shall be referred to as "Vincent's infection." This term is generally the one used by workers in the field of Medical Technology.

It is necessary for the technologist to be familiar with the etiology and clinical symptoms of the condition if for no other reason than to aid in choosing the sites for collection of material for the smears.

The etiological factors, or causative agents, were earlier thought to be only the fusiform bacillus, *Fusobacterium plauti-vincenti,* and the spirochete, *Borrelli vincentii;* and, although Rosebury, Smith and others[1] are of the opinion that there are other organisms, namely vibrios and anaerobic streptococci, present in Vincent's lesions, it is still the bacilli and the spirochetes that are looked for in large numbers in smears of Vincent's infection today.[2] They will, many times, occur in conjunction with such predisposing conditions as blood dyscrasias, general debility and severe fatigue, dietary deficiencies and poor dental hygiene. The bacterial agents of Vincent's infection can be found in normal, healthy mouths and will constitute no problem until some condition affects the tissue in such a manner as to make it favorable for invasion and growth of these organisms. Their virulence is in accord with their environment.

The clinical symptoms, very briefly, include (1) sudden onset of pain, (2) fever, (3) malaise, (4) loss of appetite, (5) headache, (6) a wedging sensation and numbness of the teeth, (7) a particularly fetid odor of the breath, and (8) tender, bleeding gums with necrosis of the affected tissue.[3,4]

Evaluation of the literature available shows a decided lack of information for the technologist as to the areas most severely

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involved with the process of infection, and without this knowledge no procedure that might be used for making smears would be adequate. Although the entire mouth is subject to the infection, in almost all cases the focal point tends to be the interdental gingival surfaces and these areas should be carefully examined when smears are made. The gingiva shows the typical blunting of the papillae and ulceration of the free margins. When the technologist is aware of this, it is unnecessary to point out that the often used "cotton swab technic" is to no avail. When a swab is superficially rubbed over the entire gingival surface and the mucous membrane of the cheek and smears are made from it, it only serves to transfer a few epithelial cells, some saliva, and almost no organisms to the slide and is hardly more instructive than a cursory glance into the patient's mouth.

Recent observations have shown us that when an instrument is used to gently scrape the interdental areas and deeply into the pockets where necrosis has taken place, large numbers of the organisms can be found and excellent smears made. These present a typical picture from a bacteriological standpoint. Care must be exercised at this point not to cause bleeding when collecting the material as an excess of blood cells means poor smears. We have used a periodontal membrane explorer, or a small curette, and found it very satisfactory. It is felt that there should be further investigation into the instruments most applicable to the task of gathering material of Vincent's infection; and it is the intention of this worker to compare, at some future time, the results obtained with a periodontal explorer, a cotton swab, a platinum loop, and a wooden applicator shaped to facilitate scraping of the gingival tissues without added trauma. The following practical procedure is outlined for making a smear:

(1) Use a chemically clean slide, ruled into several areas, preferably four, with identifying marks for each area corresponding to places in the mouth where the material was taken.

(2) Collect sample of material with an instrument suited to the job.

(3) Spread the material thinly and allow the smear to dry in the air.

Just a word about the use of a chemically clean slide. If the smear is going to be of value, it is obvious that the glass must be free from all traces of dirt and oily film which would keep the bacteria from adhering. If bacteria are washed away in the staining proces, the *true* picture is altered.

When the technologist has completed the preparation of the smears, the next consideration is the staining of them. There are almost as many methods of staining as there are textbooks of laboratory procedures, and some of them are unnecessarily involved. The only prerequisite of a stain for Vincent's organisms

is that it must stain both the fusiform bacilli and the spirochetes evenly and intensely. After air-drying the smear and fixing it in a low flame, a 2% aqueous solution of Crystal Violet can be applied for 2 minutes and then washed off with distilled water. This will demonstrate all organisms clearly. The morphology of the two closely associated organisms is interesting and should be a familiar subject with the Medical Technologist. The bacilli are spindle-shaped, usually occurring in pairs with blunted connecting ends and pointed free ends. Each rod is approximately 8-16 microns long and 0.5-1.0 microns wide. Very often it is granular in appearance. The spirochete is 8-12 microns in length and 0.3 microns in width with 3-8 shallow irregular spirals.[5,6]

The laboratory report of a Vincent's smear should be carefully worded. Since it may be used to aid and abet the clinical findings, it must leave no doubt in the clinician's mind as to exactly what was seen on the smear. If reporting the *presence* of Vincent's organisms, it would be wise to report the concentration or relative numbers of these organisms. This may be accomplished simply by using the phrase "large numbers present" to indicate that many of these microorganisms are seen in many fields throughout the smear and "small numbers present" when few microorganisms are seen in scattered fields throughout the smear. Since fusiform bacilli and spirochetes may be found in mouths from which Vincent's infection is absent, this type of report is necessary.

As part of a discussion of necrotizing ulcerative gingivitis, or Vincent's infection, I think the Medical Technologist will find the following quotation from Miller's *Textbook of Periodontia* of interest. "A special committee of the Research Commission of the American Dental Association reported the following concerning the communicability of Vincent's infection (acute necrotizing ulcerative gingivitis). In the early days Vincent's infection, when less known was thought to be highly infectious. More recently, as a result of careful research, plus the observations of many clinicians in the field, this theory has been largely discounted. On this point, Rosebury says that the etiology and pathogenesis of Vincent's infection suggest that it is not communicable and there seems to be no clear or convincing direct or other evidence of the alternative. He states that it is now known that the microorganisms responsible for the characteristic symptoms occur widely if not universally in all mouths. It is therefore unnecessary to postulate their introduction from the outside world as a prerequisite for the appearance of the symptoms. On the contrary, Vincent's infection seems to be one of a group of endogenous infections which like sub-acute bacterial endocarditis or peritonitis due to intestinal perforation, are

induced by ordinary harmless surface parasites under particular conditions. These diseases as a group, are not communicable."

SUMMARY

This paper is presented as a means of bringing to your attention the various problems that confront the Medical Technologist in the preparation of smears that might aid the clinician in his diagnosis of Vincent's infection. Much work remains to be done in the field of etiology and epidemiology of the disease and also on the actual standardization of procedure. Until such time as that work is completed, it is thought that the following aspects, in summary, are worthy of the attention of all workers in clinical and dental laboratories:

(1) Knowledge of the causative agents and of how they manifest themselves in a pathologic process.
(2) Knowledge of the areas where organisms appear in greatest concentration and of the method of collection.
(3) Knowledge of adequate staining procedures and of methods of reporting results.

REFERENCES

1. Bibby, B. G. The Role of Bacteria in Periodontal Disease. Oral Surgery, Oral Medicine, Oral Pathology, Feb., 1953, p. 318.
2. Grosby, J. S. Evaluation of the Oral Smear in Vincent's Infection. Jour. of Periodontology, 9: 85, July, 1938.
3. Miller, S. C. Textbook of Periodontia, Philadelphia, The Blakiston Company, 1950, pp. 484-488.
4. Hill, T. J. Oral Pathology, Philadelphia, Lea and Febiger, 1949, p. 328.
5. Bergey's Manual of Determinative Bacteriology, Sixth Edition, p. 581.
6. Appleton, J. L. T. Bacterial Infection in Dental Practice, Fourth Edition, Philadelphia, Lea and Febiger, 1950, pp. 540-555.

ANNOUNCEMENT FROM THE PROGRAM COMMITTEE

The following is the list of awards available:

1. The Hillkowitz award of $100.00 offered by the Denver Chemical Co. The work reported does not have to be original. Only members of ASMT are eligible for this award.
2. The Registry award of $50.00. This award is open to any registered technologist. The work reported does not have to be original.
3. The ASMT awards of $100.00, $50.00 and $25.00. Only ASMT members are eligible, and the work reported does not have to be original.
4. A parasitology award of $25.00 for the best paper on parasitology. Only ASMT members eligible.
5. The ASMT awards of $75.00 and $50.00 for state society and individual member exhibits.

Program Rules

1. The deadline date for notice of intent to submit papers is December 15.
2. The deadline date for the receipt of papers is March 15.
3. All competitive papers must be presented at the convention in person (preferably) or by proxy.
4. All papers read at the convention or presented to the program committee become the property of ASMT and may be published in the American Journal of Medical Technology only. Papers previously published elsewhere cannot be accepted. Prize papers from state contests are eligible for further awards if they have not been published.
5. All audio-visual aids and operators will be furnished by the Speakers Supplies Committee.
6. Five (5) copies of each manuscript submitted for an award must be sent to the program chairman. These must be typewritten, double-spaced, on typewriter paper. The form of the bibliography shall be as follows: Author(s) name(s), title of paper, journal in which published, volume, page, year of publication.
7. Two (2) copies of each manuscript must be submitted by those not competing for awards, subject to the above regulations.

More Program Notes

Among our speakers at the convention will be Dr. H. W. Brown, Professor of Parasitology at Columbia University.

JUST ANOTHER REMINDER THAT IF YOU INTEND TO SUBMIT A PAPER FOR THE 1954 CONVENTION, WHETHER FOR AN AWARD OR NOT, YOU MUST NOTIFY THE PROGRAM CHAIRMAN AS SOON AS POSSIBLE. DECEMBER 15 IS THE DEADLINE.

PLEASE DON'T DELAY. SEND THAT NOTICE **NOW.** YOU, TOO, MAY BE A PROGRAM CHAIRMAN SOME DAY.

ANNA BELL HAM, program chairman
1190 South Alhambra Circle
Coral Gables, Florida

FROM THE ENTERTAINMENT CHAIRMAN

A cabana luncheon has been arranged for Monday. The entertainment plans are progressing, and there will be a detailed report in the next issue.

AMONG THE NEW BOOKS

TECHNICAL METHODS AND PROCEDURES OF THE AMERICAN ASSOCIATION OF BLOOD BANKS. Burgess Publishing Company, Minneapolis, Minn., 1953. 88 pages. Price $2.00.

This is the first edition of a manual of proper blood bank procedures prepared by an appointed committee of the American Association of Blood Banks.

The stated aim of the manual is "to assure the proper collection and

processing of blood from healthy donors in all laboratories, large or small" by outlining methods that are considered to be the most practical and satisfactory. The reviewer considers that an excellent beginning has been made toward achieving this aim, but that the final success will depend upon the extent that thoughtful and careful readers immediately contribute their ideas and suggestions for editorial and technical improvements in future editions.

The manual contains considerable information not previously available in a single pamphlet or book. An adequate table of contents and index made subject finding relatively easy. Printing is on one side of 8½" x 10¾" pages only leaving a side free for additional notes and corrections.

The manual should be welcomed by persons that are interested in bringing their own practices into line with those considered by experts to be "proper" and to those that have an interest in contributing towards raising the standards for reagents and practices in blood transfusion services.

HORMONAL FACTORS IN CARBOHYDRATE METABOLISM. Vol. VI. Chairman of Ciba Foundation Colloquium on Endocrinology, Professor C. H. Best, C.B.E., M.D., F.R.C.P., F.R.S. Department of Physiology, University of Toronto, with 28 contributors. Little, Brown & Co., Boston, 1953. 24 articles with discussions. 350 pages. $6.75.

In six parts, concerned with the Enzyme Systems in Carbohydrate metabolism, Hormonal Control of the Interconversion of Carbohydrate, Protein, and Fat, the influence of Hormones on Carbohydrate metabolism, these articles give the results of well-controlled investigation, this volume is beyond the field of the average medical technologist, but will be of distinct aid to the biological chemist.

RESPIRATORY DISEASES AND ALLERGY by Josef S. Smul, M.D., member N. Y. Academy of Sciences. Medical Library Company, New York, 1953. 80 pages. $2.75.

A treatise on a relationship of allergy to the various respiratory diseases. Inasmuch as the book is on diagnosis, prognosis, and treatment, it does not relate to the field of medical technology.

STANDARD METHODS OF CLINICAL CHEMISTRY. Volume I, by the American Association of Clinical Chemists, Miriam Reiner, editor, director, Chemistry Laboratory, Gallinger Municipal Hospital, Washington, D. C. Academic Press, Inc., New York, 1953. 142 pages. $4.50.

This book can be described as a series of some of the most widely used tests in the chemistry laboratory. Each determination is submitted by the biochemist in charge of a department as the technique of his choice. Results have been checked by others in order to reach a conclusion that the method is a good choice. Underlying principles, reagents, tests which reveal pitfalls, all are given together with the usual procedures and conclusions. There is little new information included. However, the book was purposely designed to allow for flexibility.

LIVING WITH A DISABILITY: by Howard A. Rusk, M.D., Director of Physical Medicine and Rehabilitation, New York University, Bellevue Medical Center, New York. The Blakiston Company, New York, 1953. 207 pages. 282 photographs and line drawings. $3.50.

Although it would not be used in the clinical laboratory, and would definitely be of more interest to the Occupational or Physical Therapist than to the Medical Technologist, there is the consideration of the potential we find in the so-called physically handicapped person who might find a place in the laboratory. This book was written primarily to help the handicapped person to become self-reliant. The many illustrations show graphically how the many new appliances can be used to this end, at home or at work.

THE GIRL IN THE WHITE COAT: Helen Wells. Julian Messner, Inc., 8 West 40th St., New York 18. 184 pp. $2.50.

This is a short, lively novel, beginning with the heroine about to be gradu-

ated from high school, taking her through four years of college and hospital training to achieve her M. T. (ASCP) certificate and on to her first position. Good training and high professional standards are stressed with acknowledgment to Dr. Ralph E. Miller and Evelyn Jardine of Hitchcock Memorial Hospital, Hanover, N. H., Dr. Montgomery, Mrs. Drummond, Rose Matthaei and Frieda Claussen, names familiar to all of us.

Romance and mystery are here also, all told simply so that it should interest girls and boys of high school age. One lamentable error on pp. 118 and 119 concerns the Rh types of the parents of an erythroblastic child and the transfusion given to the infant. It is hoped that this will be corrected.

ELSA S. KUMKE.

ABSTRACT

The paper PREPARATION OF MATERIAL FROM HEMATOPOIETIC ORGANS FOR MORPHOLOGIC STUDY by Lawrence Berman, M. D., Professor of Hematopathology, Wayne University, College of Medicine, Detroit, Michigan, and Elsa S. Kumke, B. S., M.T. (ASCP) presented by Mrs. Kumke at the Louisville convention will not be published in THE AMERICAN JURNAL OF MEDICAL TECHNOLOGY. Most of the technics have been described elsewhere and most of the lantern slides used for illustrations.

For those who are especially interested we refer you to two other publications by Dr. Berman:

A REVIEW OF THE METHODS FOR ASPIRATION AND BIOPSY OF BONE MARROW. The American Journal of Clinical Pathology, 23: 385, April 1953. Technical Bulletin of the Registry of Medical Technologists, 23: 47, March 1953.
MALIGNANT LYMPHOMAS. THEIR CLASSIFICATION AND RELATION TO LEUKEMIA. Blood VIII: 195, March 1953.

REMOVING STAINS IN THE LABORATORY

A. T. BARTHOLOMEW, M.T. (ASCP), Houston, Texas

For more detailed information on the removal of stains see the PHYSICIAN'S DESK REFERENCE. We have tried most of those listed below in the laboratory. WARNING: DO NOT USE ON NYLON or other synthetic fabrics unless you have a small piece of the same material to use as a preliminary check (then make up your mind whether the stain or the hole is more desirable). These have been tested on COTTONS.

ACIDS: Acid stains are treated with ammonia or sodium bicarbonate. They are then thoroughly washed.

ANILINE DYES (Methylene Blue): Lye solution or a dilute hydrochloric acid solution. Either method must be followed by a thorough rinsing with water. Very resistant dyes must be soaked for several hours in a 1-1000 permangenate solution. Rinse and treat with an oxalic acid solution.

BALSAM OF PERU (VERY HARD TO REMOVE): Fresh stains may be treated with chloroform, acetic acid or amyl alcohol. Old stains should first be softened with benzyl benzoate.

CANADA BALSAM: Treat with ether, amyl alcohol, chloroform, oil of turpentine, carbon tetrachloride, carbon bisulfide, and tuluol; in most instances alcohol, acetone, methanol, acetic ether, and benzine will do the trick.

CHROMIC ACID (provided there is anything left from which to remove the stain!): Dilute sulfurous acid in watery solution or concentrated sodium thiosulfate solution mixed with a few drops of sulfuric acid. When the fabric is suitable, the acid may be precipitated into lead chromate with lead acetate. After this is washed in distilled water, it may be dissolved with lye.

COFFEE or COCOA: Wash in concentrated salt water. Rinse thoroughly with water. Sometimes the stain will have to be softened with glycerin.

COPPER SULFATE: Moisten the stains with a 30% solution of potassium iodine or a 10% solution of acetic acid, followed by a lukewarm solution of 10% sodium chloride. Potassium Cyanide CAN be used.

GOLD (colloidal): Use 20% potassium cyanide.

HEMOGLOBIN: Fresh blood stains should be washed in lukewarm (never HOT) water. Older stains should be softened with borax solution, dilute ammonia or tincture of green soap. Finally this is treated with a 2% solution of oxalic acid. Pepsin with hydrochloric acid or other enzyme preparations may be used for resistant stains.

INK (Methyl Violet): This may be removed from the skin with glycerin, acetic or citric acid. Fabrics require similar applications. Resistant stains must be treated as TAR COLORING products.

IODINE: Hands or clothes are moistened with ammonia or sodium thiosulfate solution.

LIPSTICK: Soften with glycerin. Then soak in mild soap and water solution or use a detergent.

MILDEW: Cover the stain with ammonia water, then saturate with 20% solution of oxalic acid (use a cotton pad). Rinse thoroughly in water. They may also be removed by wetting with dilute hydrochloric acid followed by hydrogen peroxide.

PICRIC ACID: Dissolve the stain in an alkaline sulfate for about a minute, then wash thoroughly with soap and water. Fresh stains may be covered with a paste made up of magnesium carbonate and water. Rub paste into the stain and rinse in clear water.

POTASSIUM PERMANGENATE: Dilute sulfurous acid or dilute hydrochloric acid, oxalic acid, hyposulfite, or alkaline sulfates. For susceptible fabrics (and marble) which cannot be treated with acids, moisten the stain with ammonium sulfate solution. Wash this away. The manganese sulfate formed is dissolved with a 10% solution of potassium cyanide.

RED WINE: Sodium perborate or hydrogen peroxide solution.

RUST, IRON SALTS: Ferric chloride, tinctures of iron salts, and rust yield to treatment a 10% zinc chloride solution of oxalic acid to which about 5% glycerin is added, with hot sodium hyrophosphate solution. Weak watery solutions of ammonium fluoride (lead, gutta-percha, paraffin, or wooden vessels should be used. White fabric may also be treated with a hydrosulfite (sodium hydrosulfite may be used). This is sprinkled on the stain which is then moistened. In all instances the material should be thoroughly rinsed with water.

SILVER NITRATE: The simplest treatment is to moisten the stain with iodine. Then wash out immediately with cool or lukewarm water. Fresh stains can be quickly removed with a 10% solution of potassium cyanide, followed immediately by bichloride of mercury and a thorough rinsing with water. A 10% solution of potassium iodide may be used, with the resulting yellow stains being removed with thiosulfate solution.

TAR COLORING PRODUCTS: Dried stains should be softened with warm oil, then benzol, zylol, chloroform, carbon tetrachloride, trichlorethyl, or tetralin for dissolving the stain. (A blotter should be placed under the stain to absorb the excess.) The fabric is then washed with a tincture of green soap or with soap and water. Rinse.

URINE: Stains may be removed by wetting with dilute hydrochloric acid, followed by hydrogen peroxide.

X-RAY DEVELOPER: Wipe off with a moist zonite or chlorox swab. Rinse.

Ed. Note: If you have a better "mouse trap," please advertise it. Any pointers to help your fellow technologists to make the laboratory a more "efficient entity" will be appreciated. Send to AJMT, 25 Hermann Professional Bldg., Houston 25, Texas.

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